REMARKS

Claims 22-25, 28, 29, 33-43, 46, 47, 59, 62, 64, 80-91, 94-98, 100, 101, 104, 106, 108-111, 116, and 117 are currently pending. Claims 22-25, 28, 29, 33-40, 43, 46, 47, 59, 62, 64, 87-89, 96, 101, 106, 108-111, 116, and 117 are under examination. Claims 41, 42, 80-86, 90, 91, 94, 95, 97, 98, 100, and 104 are withdrawn from consideration. The amendments to the claims do not introduce new subject matter and were made to more clearly define the claimed subject matter. A complete set of the pending (examined and withdrawn) claims is provided for the Examiner's convenience.

Attached hereto is a "VERSION WITH MARKINGS TO SHOW CHANGES MADE" to detail the amendments made to the claims.

35 USC §112 rejections

Claims 46, 88-89, 93, 96, 100 and 117 were rejected under 35 USC §112, second paragraph, as being indefinite for failing to point out and distinctly claim the subject matter which applicant regards as the invention. In response, Applicant has amended the claims to more clearly define the subject matter. Applicant submits that the claims, as amended, clearly define the subject matter and respectfully requests withdrawal of these rejections.

35 USC §103 rejections

Widder, Connelly, and Abram

Claims 22-25, 28-29, 33, 36-38, 48, 51, 59-62, 64, 66, 69, 101, 106, and 108-115 are rejected under 35 USC §103(a) over *Widder* et al. (EP 016,552) in view of *Connelly* et al. (U.S. Patent No. 5,422,277) in further view of *Abram* et al. (U.S. Patent No. 4,497,900). Applicants traverse this rejection and respectfully request reconsideration.

Widder discloses a method for coarse separation of blood cells through use of microspheres having protein A associated with the outer surfaces thereof. However, *Widder* fails to teach a first antibody that binds a second antibody. *Widder* also fails to teach detecting a specific target cell at a sensitivity of one target cell per 100 or more total cells. *Connelly* does not overcome the deficiencies of *Widder*, as *Connelly* also fails to teach a first antibody that binds a second antibody. Further, *Connelly* fails to teach

detecting a specific target cell at a sensitivity of one target cell per 100 or more total cells. *Connelly* merely directs the reader to a cell fixative composition for fixing the internal components of a cell without disrupting the cell surface components.

Moreover, the combination of *Widder* and *Connelly* fails to teach all the elements of the claimed invention. *Widder* and *Connelly* are deficient in teaching the detection of a specific live target cell present at a concentration of one target cell per 100 or more total cells. The combination of *Widder* and *Connelly* would result in a fixed population of cells that are no longer live, in which some cells are labeled with beads coated with a single species of antibody. The claims, as amended, recite the detection of live target cells that are present at a concentration of 1 target cell per 100 total cells. Neither *Widder* nor *Connelly* teach detecting live cells at such a concentration.

Abram does not cure the deficiencies of *Widder* and *Connelly* and is further removed from the concept of the claimed invention. Thus, the combination of *Widder, Connelly,* and *Abram* fails to teach all the elements of the claimed invention. *Abram* does not teach a first antibody that is directed against second antibody or antibody fragment that is directed against a cell membrane structure. Instead of detecting a membrane structure on an intact, live target cell, the antibodies described by *Abram* are directed to antigens from lysed bacteria that have been absorbed onto plastic beads. Furthermore *Abram* is deficient in teaching the coating of magnetic particles with a first antibody or antibody fragment directed against a second antibody. *Abram* is also deficient in teaching the detection of target cells at the claimed concentration. *Abram* specifically teaches increasing the sensitivity of the assay by lysing bacterial cells to release the antigens to be detected, thereby increasing the number of antigenic sites available for binding to gonococcal antibody (see column 2, lines 52-56). Thus, *Abram* is also deficient in teaching the detection of a specific live target cell.

The Examiner asserts that the sensitivity levels appear to be achieved by optimization procedures, and that it has long been settled to be no more than routine experimentation for one to discover an optimum value of a result effective variable. However, as none of the cited references, either alone or in combination, teach all of the elements of the claimed invention, there is no motive for one of ordinary skill in the art to further modify or experiment with the teachings of the references to achieve "optimum

values". Applicants submit that, in order to change the cited teachings into the claimed invention, much more than mere discovery of an "optimum value of a result effective variable" would be required. Additionally, the Examiner has provided no indication of just what the result effective variable would be, or how one would go about optimizing it based on the teachings of *Widder*, *Connelly*, and *Abram*, to achieve the claimed invention.

Therefore, *Widder*, *Connelly*, and *Abram*, taken alone or in combination, do not teach all the elements of the claimed invention. Therefore, all of the criteria for establishing a *prima facie* case of obviousness rejection have not been met. Applicants respectfully request this rejection be withdrawn.

Widder, Connelly, Abram, and Forrest

Claims 22, 46-48 and 106 are rejected under 35 USC §103(a) over *Widder* et al. in view of *Connelly* et al., in further view of *Abram* et al. and *Forrest* et al. Applicants traverse this rejection and respectfully request reconsideration.

The Examiner maintains that it would have been obvious to incorporate the antibodies, buffers, beads, and reagents in the methods of *Widder, Connelly* and *Abram* into a test kit such as that taught by *Forrest* because test kits are conventional and well known in the art.

Forrest does not overcome the deficiencies of *Widder, Connelly* and *Abram*. As discussed above, the combination of *Widder, Connelly* and *Abram* fails to teach a first antibody that binds a second antibody. Also, the combination of *Widder, Connelly* and *Abram* fails to teach detecting a specific live target cell at a sensitivity of one target cell per 100 or more total cells. *Forrest* does not teach a first antibody that binds a second antibody and does not teach a detecting a specific live target cell at a sensitivity of one target cell per 100 or more total cells. As such, a combination of *Widder, Connelly, Abram* and *Forrest* would not achieve the instant invention. Applicants respectfully request this rejection be withdrawn.

Widder, Connelly, Abram, Kemmner, and Holmes

Claims 34-35, 39-40, 43, 71-72, 75, 87-89, 92-93, 96 and 116 are rejected under 35 USC §103(a) over *Widder* et al. in view of *Connelly* et al., and further in view of *Abram* et al., *Kemmer* et al. and *Holmes* et al. Applicants traverse this rejection and respectfully request reconsideration.

Kemmner and *Holmes* do not overcome the deficiencies of *Widder, Connelly* and *Abram*. As discussed above, the combination of *Widder, Connelly* and *Abram* fails to teach a first antibody that binds a second antibody. Also, the combination of *Widder, Connelly* and *Abram* fails to teach detecting a specific live target cell at a sensitivity of one target cell per 100 or more total cells. *Kemmner* is cited for teaching isolation of tumor cells using magnetic beads coated with monoclonal antibodies. *Holmes* is cited for teaching a method of separating hematopoietic progenitor cells from a mixed population using microbeads coated with antibodies.

Kemmner teaches their method as detecting only 40% of target cells present at a concentration of 73% of total cells (see page 199, second column). *Kemmner* teaches that steric hindrance and resulting reduced accessibility of epitopes for binding the antibody-coated beads, could be responsible for the reduced level of detection as opposed to the better results obtained by immunofluorescent assay on frozen tissue sections. Thus, *Kemmner* fails to teach detecting a specific live target cell at a sensitivity of one target cell per 100 or more total cells. Thus, one of ordinary skill in the art, upon reading *Kemmner*, would not be motivated to use antibody-coated beads for separation or identification of target cells present at a concentration of 1% or less of the total cell population.

Holmes is directed to separating blood cells from a blood or bone marrow sample, which is specifically excluded from the instant claims. Thus, *Holmes* cannot be seen to provide any guidance or motivation for modifying any of the previously described prior art to achieve the instant invention.

As such, the combination of *Widder, Connelly, Abram, Kemmner,* and *Holmes* fails to achieve the instant invention. Applicants respectfully request this rejection be withdrawn.

Widder, Connelly, Abram, Kemmner, and Forrest

Claim 117 is rejected under 35 USC §103(a) over *Widder* et al. in view of *Connelly* et al., in further view of *Abram* et al., and in further view of *Kemmner* et al. and *Forrest* et al. Applicants traverse this rejection and respectfully request reconsideration.

The Examiner maintains that it would have been obvious to incorporate the antibodies, buffers, beads, and reagents in the methods of *Widder, Connelly, Abram,* and *Kemmner* into a test kit arrangement such as that taught by *Forrest* because test kits are conventional and well known in the art.

Forrest does not overcome the deficiencies of *Widder, Connelly, Abram* and *Kemmner*. As discussed above, *Forrest* does not teach a first antibody that binds a second antibody and does not teach a detecting a specific live target cell at a sensitivity of one target cell per 100 or more total cells. As such, a combination of *Widder, Connelly, Abram, Kemmner* and *Forrest* would not achieve the instant invention. Applicants respectfully request this rejection be withdrawn.

In view of the remarks presented herein, Applicants respectfully submit that the claims are in condition for allowance. Notification to that effect is earnestly solicited. If prosecution of this case could be facilitated by a telephonic interview, the Examiner is encouraged to call the undersigned.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Please cancel claims 48, 51, 60, 61, 66, 67, 69, 71-75, 92, 93, 99, 102, 103, 105, and 112-115 and amend claims 22, 39, 46, 59, 62, 64, 80, 83, 84, 87, 88, 89, 91, 96-98, 100, 101, 104, and 117 as follows:

22. (Four Times Amended) A method for detecting a specific living target cell in a cell suspension of a mixed cell population, in a fluid system containing a mixed cell population, or in a cell suspension prepared from a solid tissue, at a sensitivity of one target cell per 100 or more total cells, with the exception of normal and malignant hematopoietic cells in blood and bone marrow, the method comprising the steps of:

a. coating paramagnetic particles or beads with a first antibody or antibody fragment directed against a second antibody or antibody fragment;

b. incubating the second antibody or antibody fragment with the cell suspension to bind the second antibody or antibody fragment with the target cell, thereby creating a cell mixture, wherein the second antibody or antibody fragment is directed against a membrane structure specifically expressed on the target cell and not on a non-target cell in the cell mixture;

c. washing the cell mixture to remove unbound second antibody or antibody fragment;

d. mixing the coated paramagnetic particles or beads with the washed cell mixture;

e. incubating the washed cell mixture and the coated paramagnetic particles under gentle rotation at about 4°C until target cell-bead rosettes are formed; and

f. visually detecting the target cell-bead rosettes after incubation.

39. (Twice Amended) The method of claim 22, wherein the second antibody or antibody fragment is directed against fibronectin receptor, β-integrin, vitronectin receptor, αγβ3-integrin, P-seletin including GMP-140, CD44-variants, N-CAM including CD-56, E-cadherin, Le^y, carcinoembryonic antigen or CEA, EGF receptor, c-erbB-2

including HER2, transferin receptor, TNF-receptor, high molecular weight antigen, p95-100, sarcoma antigens including TP-1 and TP-3 epitope, Mv 200kD, Mv160kD, MOC-31 epitope including cluster 2 epithelial antigen, MUC-1 antigen including DF3-epitope and gp290kD, prostate high molecular antigen, TAG 72, bladder carcinoma antigen, Mv 48kD colorectal carcinoma antigen, lung carcinoma antigen Mv 350-420kD, Mel-14 epitope, β_2-microglobulin, Apo-1 epitope, or pan-human cell antigen.

46. (Four Times Amended) A kit for performing the method of claim 22, the kit comprising:

a. a first antibody, wherein said first antibody is a specific monoclonal antibody or antibody fragment directed against a second antibody or antibody fragment, said first antibody [capable of] effective for coating a paramagnetic particle or bead without removing its antigen-binding ability;

b. a paramagnetic particle or bead; and

c. [a] the second antibody, wherein said second antibody is a specific monoclonal antibody or antibody fragment directed against an antigen or a receptor within or on the target cell;

wherein said second antibody or antibody fragment is conjugated to a detectable label.

59. (Twice Amended) The method of claim [48] 22, wherein the second antibody or antibody fragment directed against a membrane structure specifically expressed on the target-cell is a murine or a human antibody or fragment thereof.

62. (Four Times Amended) The method of claim [48] 22, wherein [when the ratio of target cell/total cells in the cell mixture is ≤ 1%,] the method further comprises after incubating, applying a magnetic field to separate out the target cell-bead rosettes.

64. (Twice Amended) The method of claim [48] 22, wherein visually detecting includes counting the target cell-bead rosettes using a microscope or a cell or particle counting device.

80. (Amended) [A] The method according to claim 22 further comprising after incubating; detecting a second antigen of the target cell by adding a second labeled monoclonal antibody directed to the second antigen to the cell suspension; and quantitating the amount of labeled second monoclonal antibody bound to the rosettes.

83. (Amended) [A] The method according to claim 22, further comprising before mixing; prelabeling the target cells with a labeled second monoclonal antibody to second antigen on the target cell; and after incubating, quantitating the amount labeled second monoclonal antibody bound to the rosettes.

84. (Amended) [A] The method according to claim 22, further comprising after incubating, applying a magnetic field to separate out the target cell bead rosettes; and detecting target cells specific genes at the DNA, mRNA or protein level.

87. (Three Times Amended) A method for detecting living tumor cells in a cell suspension of mixed cell population or in a cell suspension prepared from a solid tissue, at a sensitivity of one target cell per 100 or more total cells, with the exception of normal and malignant hematopoietic cells in blood and bone marrow, comprising:

a) coating paramagnetic particles with a first antibody or fragment directed against a second tumor-specific monoclonal antibody or fragment;

b) incubating the second tumor specific antibody or antibody fragment with the cell suspension to allow the second tumor specific antibody or antibody fragment to bind the tumor cells;

c) washing the cell suspension to remove unbound second antibody or antibody fragment;

d) mixing the coated paramagnetic particles with the cell suspension;

e) incubating the mixture at about 4°C under gentle rotation until tumor cell-bead rosettes are formed; and

f) visually detecting the tumor cell-bead rosettes.

88. (Amended) [A] The method according to claim 87 further comprising after incubating; applying a magnetic field to the mixture to separate out the tumor cell-bead rosettes.

89. (Amended) [A] The method according to claim 87, wherein the tumor-specific monoclonal antibody is specific for tumor antigens comprising a growth factor receptor, an oncogene product expressed on the membrane of a malignant cell, an adhesion membrane molecule, an MDR protein, breast, ovarian or lung carcinoma cells; melanoma, sarcoma, glioblastoma or cancer cells of the gastrointestinal tract; melanoma, sarcoma, glioblastoma or cancer cells of the genitourinary tract; or melanoma, sarcoma, glioblastoma or cancer cells of the reticuloendothelial system.

91. (Amended) [A] The method according to claim 87 further comprising, after incubating; detecting a second antigen on the tumor cell by adding a labeled second monoclonal antibody specific for the second antigen to the cell suspension; and quantitating the amount of labeled second monoclonal antibody bound to the tumor cell-bead rosettes.

96. (Amended) [A] The method according to claim [93] 87, wherein the mixture is incubated for about 30 minutes.

97. (Amended) [A] The method according to claim 91, wherein the tumor cell-bead rosettes are quantitated by counting them using a microscope or a cell or particle counting device.

98. (Amended) [A] The method according to claim 91 further comprising after quantitating; culturing the tumor cell-bead rosettes in a growth medium until a cell culture is established.

100. (Amended) [A] The method according to claim 97, wherein the labeled third monoclonal antibody is labeled with floureseeine, a radioactive compound, biotin or an enzyme.

101. (Amended) [A] The method according to claim [48] 22, further comprising after incubating; applying a magnetic field to the mixture to separate out the target cell-bead rosettes; and detecting target cell specific genes.

104. (Amended) [A] The method according to claim [48] 22 further comprising, after [incubating] step (e); applying a magnetic field to the mixture to separate out target cell-bead rosettes; and culturing the target cell-bead rosettes in a growth medium to establish a cell culture.

117. (Amended) A kit for performing the method of claim 22, the kit comprising:

a. a first antibody, wherein said first antibody is a specific monoclonal antibody or antibody fragment directed against a second antibody or antibody fragment, said first antibody [capable of] effective for coating a paramagnetic particle or bead without removing its antigen-binding ability;

b. a paramagnetic particle or bead; and

c. [a] the second antibody, wherein said second antibody is a specific monoclonal antibody or antibody fragment directed against an antigen or a receptor within or on the target cell, wherein the second antibody or antibody fragment is directed against fibronectin receptor, β-integrin, vitronectin receptor, $\alpha\gamma\beta3$-integrin, P-seletin including GMP-140, CD44-variants, N-CAM including CD-56, E-cadherin, Le^y, carcinoembryonic antigen or CEA, EGF receptor, c-erbB-2 including HER2, transferin receptor, TNF-receptor, high molecular weight antigen, p95-100, sarcoma antigens including TP-1 and TP-3 epitope, Mv 200kD, Mv160kD, MOC-31 epitope including cluster 2 epithelial antigen, MUC-1 antigen including DF3-epitope and gp290kD, prostate high molecular antigen, TAG 72, bladder carcinoma antigen, Mv 48kD colorectal carcinoma antigen, lung carcinoma antigen Mv 350-420kD, Mel-14 epitope, β_2-microglobulin, Apo-1 epitope, or pan-human cell antigen;

wherein said second antibody or antibody fragment is conjugated to a detectable label.